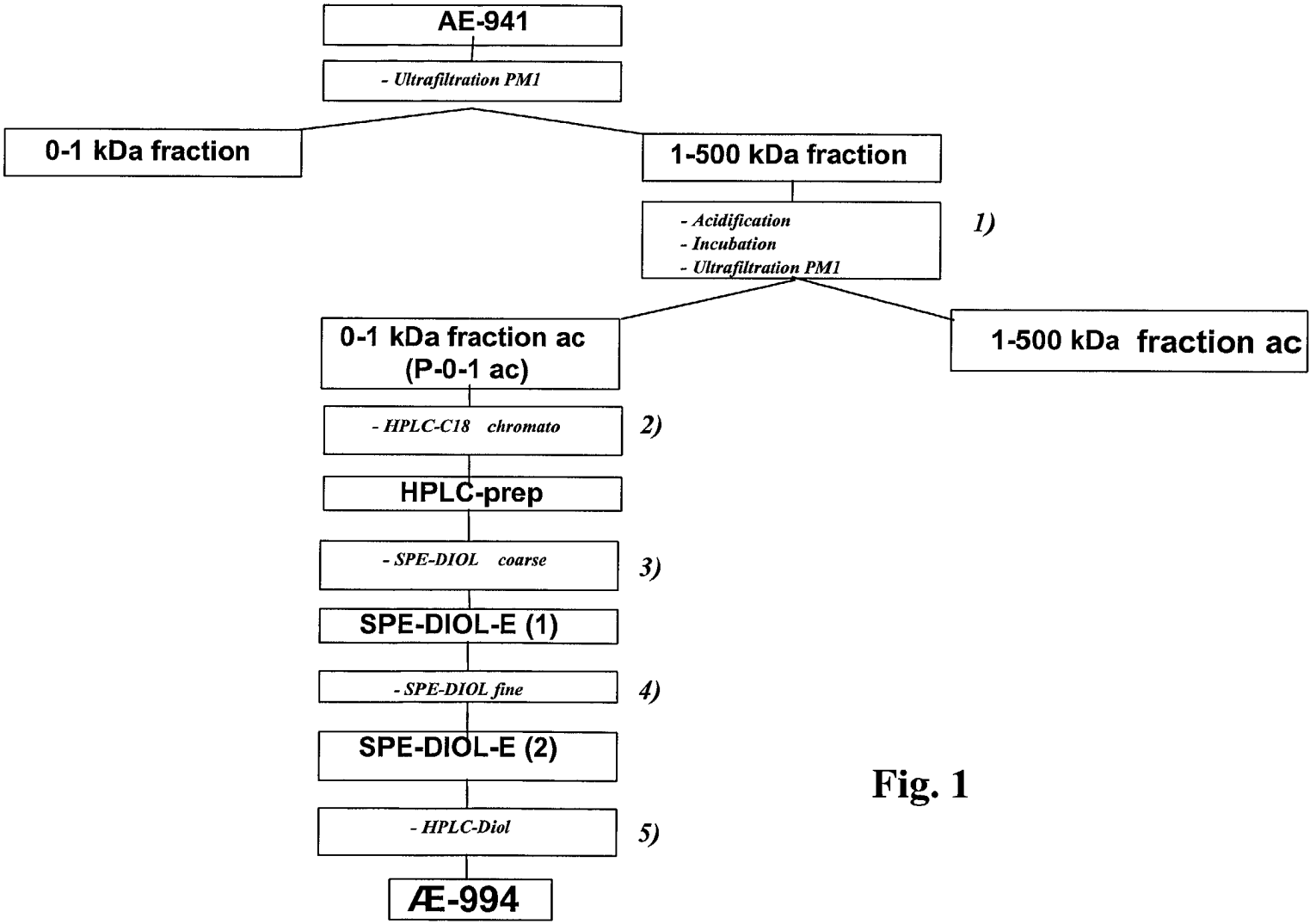

AE-941
- Ultrafiltration PM1
0-1 kDa fraction
1-500 kDa fraction
- Acidification
- Incubation
- Ultrafiltration PM1
1)
0-1 kDa fraction ac (P-0-1 ac)
1-500 kDa fraction ac
- HPLC-C18 chromato
2)
HPLC-prep
- SPE-DIOL coarse
3)
SPE-DIOL-E (1)
- SPE-DIOL fine
4)
SPE-DIOL-E (2)
- HPLC-Diol
5)
Æ-994

Fig. 1

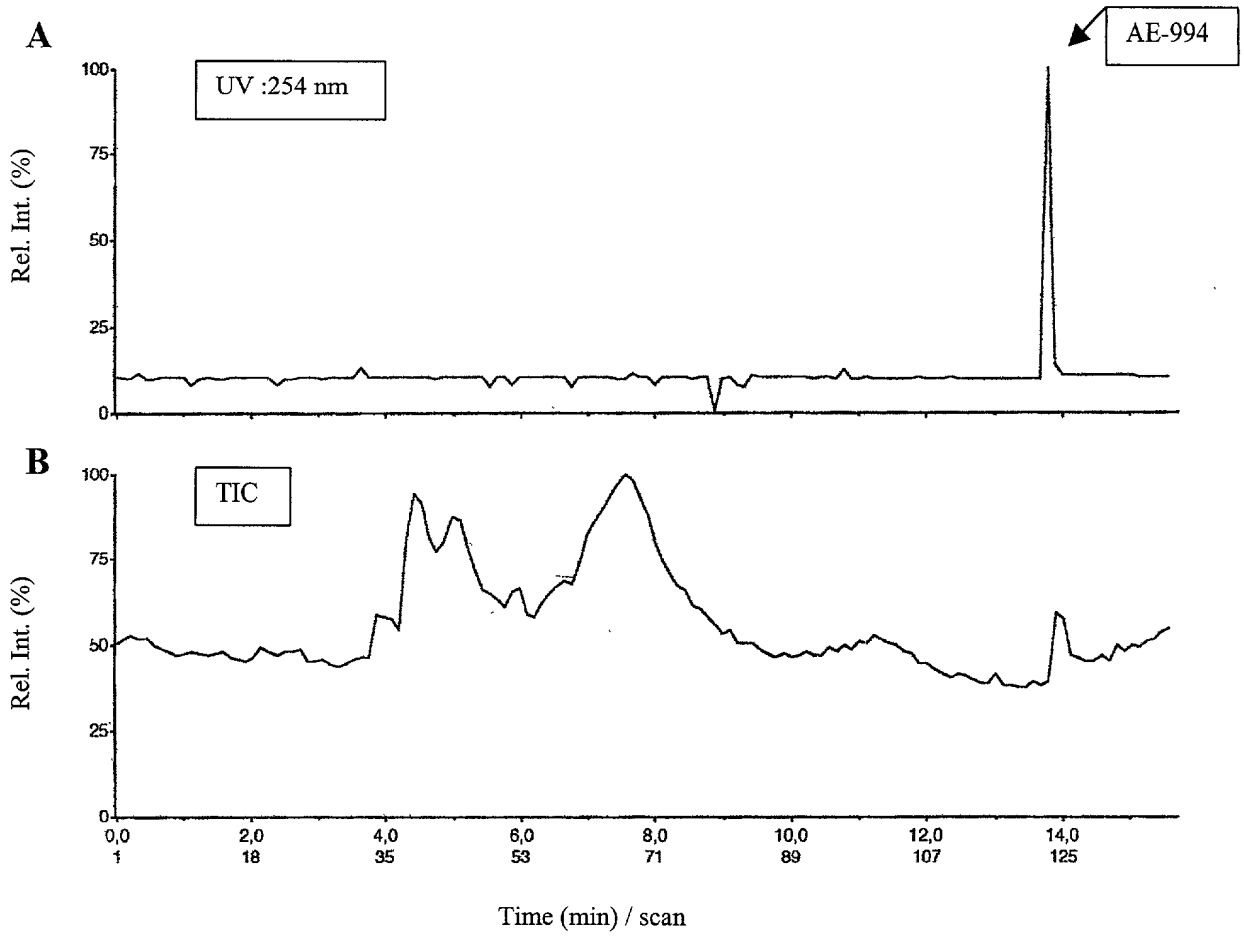

A
AE-994
UV :254 nm
Rel. Int. (%)
100
75
50
25
0
B
TIC
Rel. Int. (%)
100
75
50
25
0
0,0
1
2,0
18
4,0
35
6,0
53
8,0
71
10,0
89
12,0
107
14,0
125
Time (min) / scan

Fig. 2

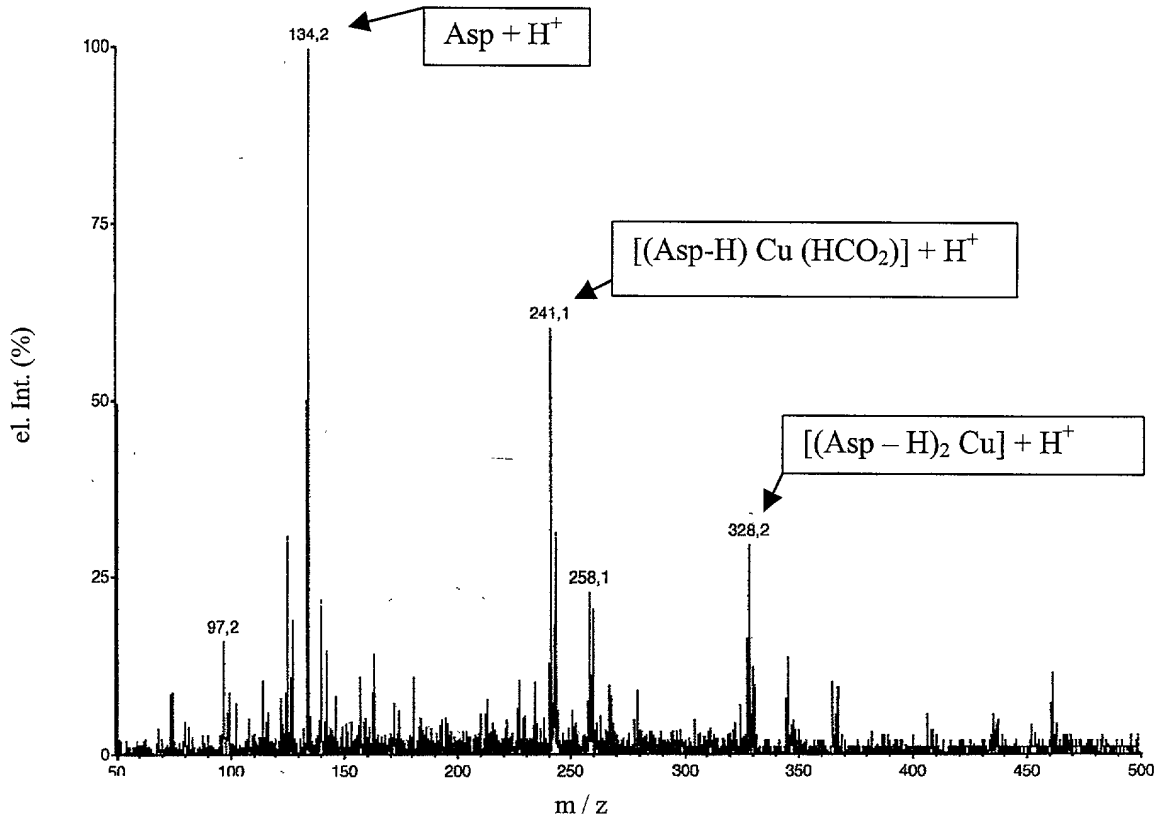

Asp + H+
134,2
[(Asp-H) Cu (HCO2)] + H+
241,1
[(Asp – H)2 Cu] + H+
328,2
258,1
97,2
el. Int. (%)
m / z
100
75
50
25
0
50
100
150
200
250
300
350
400
450
500

Fig. 3

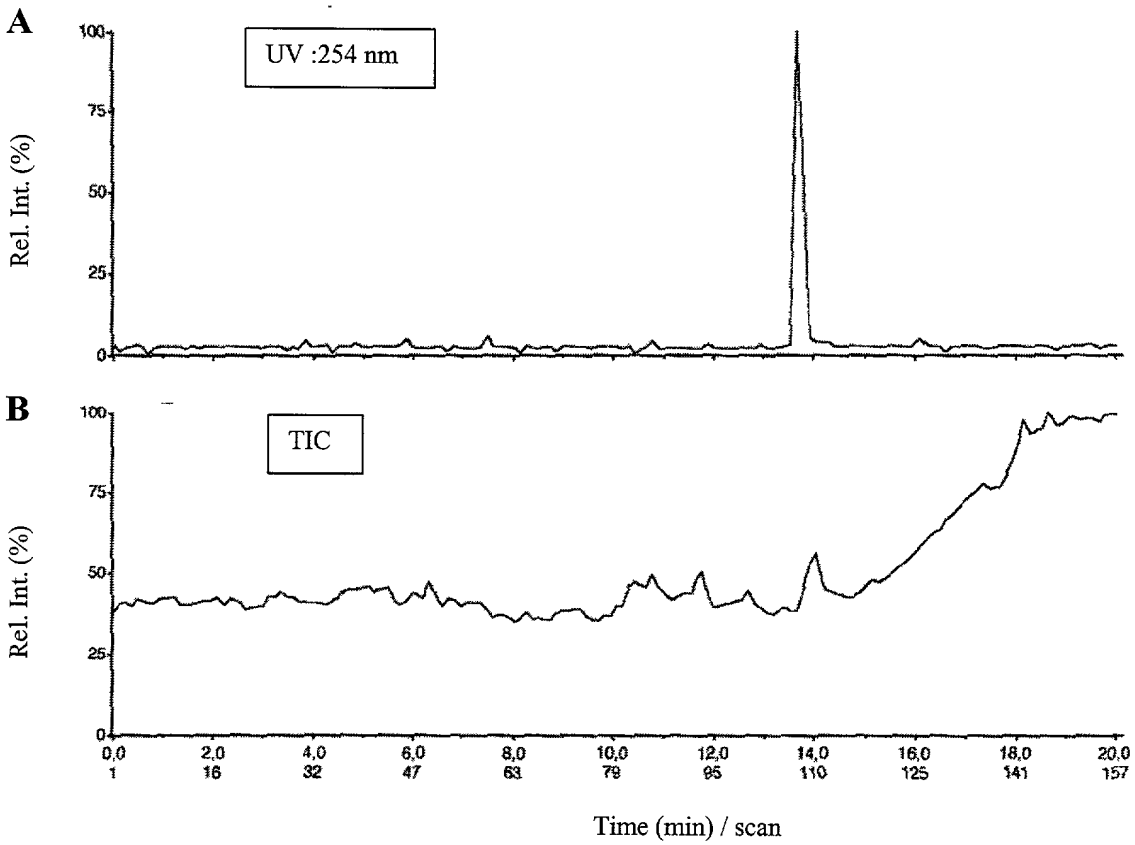

A
UV :254 nm
Rel. Int. (%)
100
75
50
25
0
B
TIC
Rel. Int. (%)
100
75
50
25
0
0,0
1
2,0
16
4,0
32
6,0
47
8,0
63
10,0
79
12,0
95
14,0
110
16,0
125
18,0
141
20,0
157
Time (min) / scan

Fig. 4

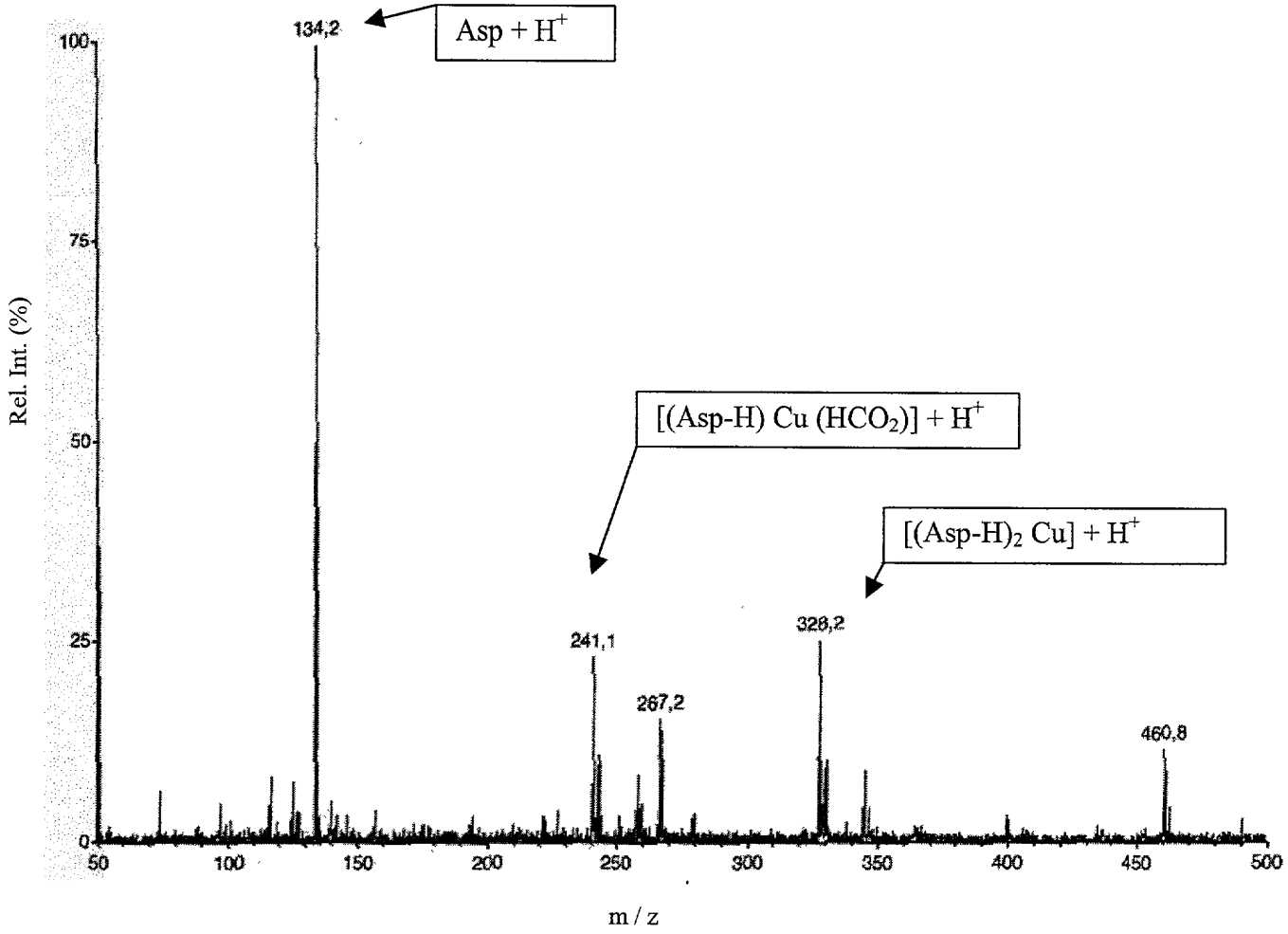
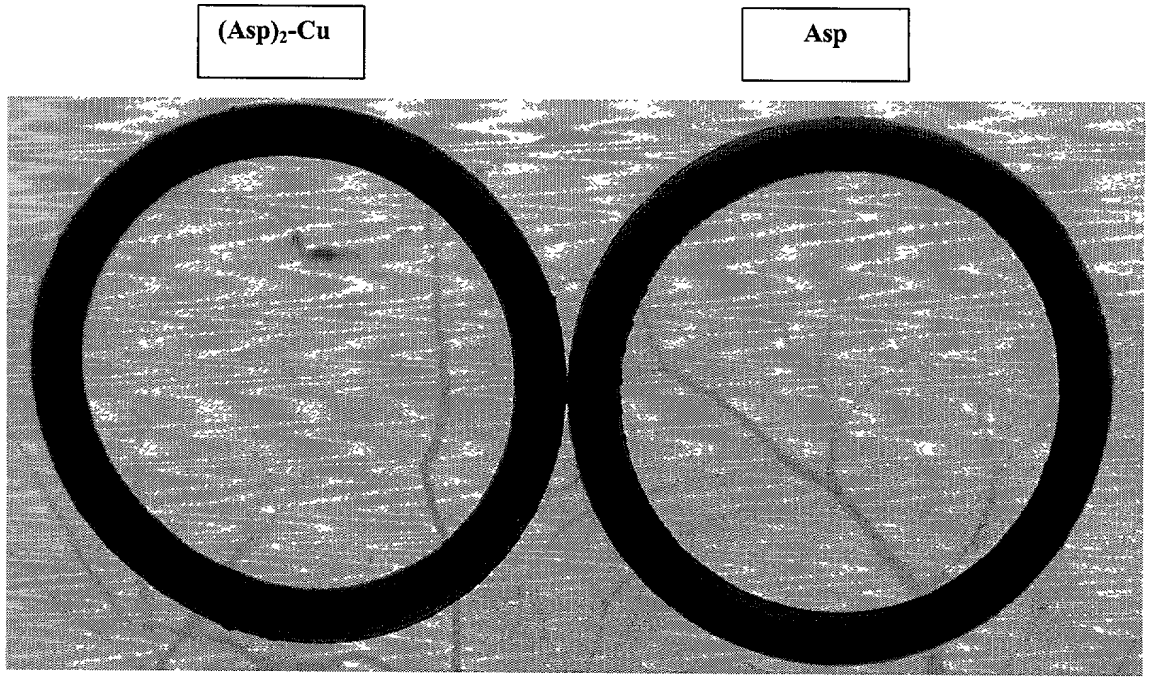
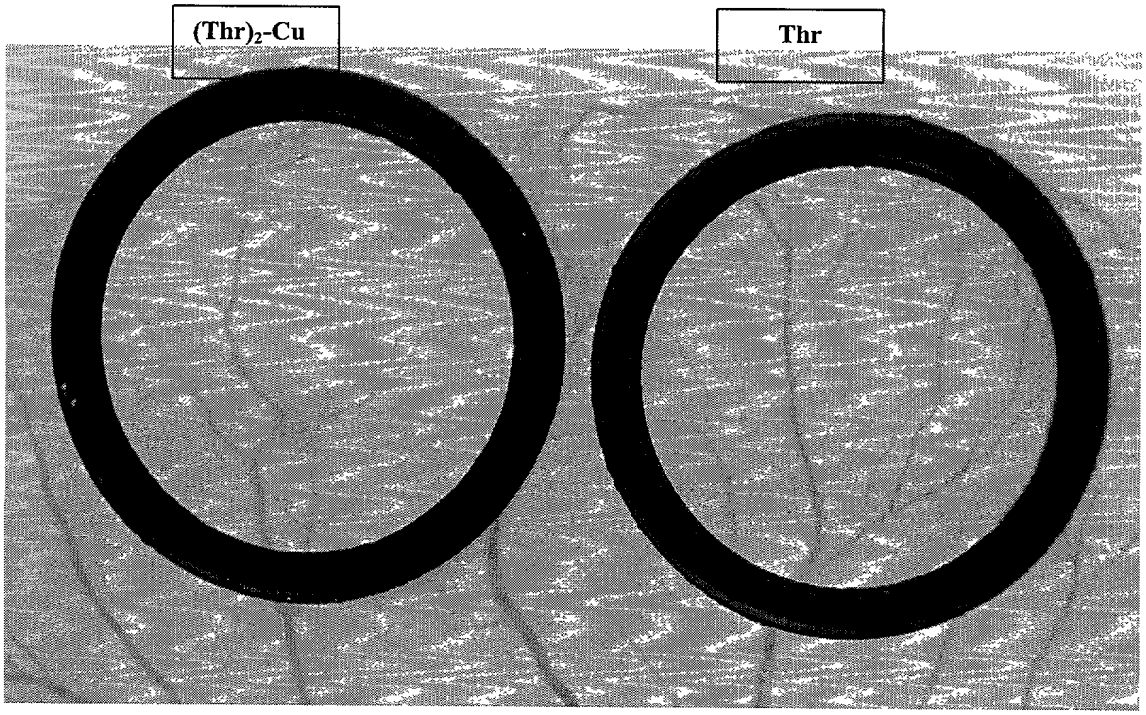

Asp + H+
134,2
[(Asp-H) Cu (HCO2)] + H+
[(Asp-H)2 Cu] + H+
241,1
267,2
328,2
460,8
Rel. Int. (%)
m / z
100
75
50
25
0
50
100
150
200
250
300
350
400
450
500

Fig. 5



(a)



(b)

FIG. 6A

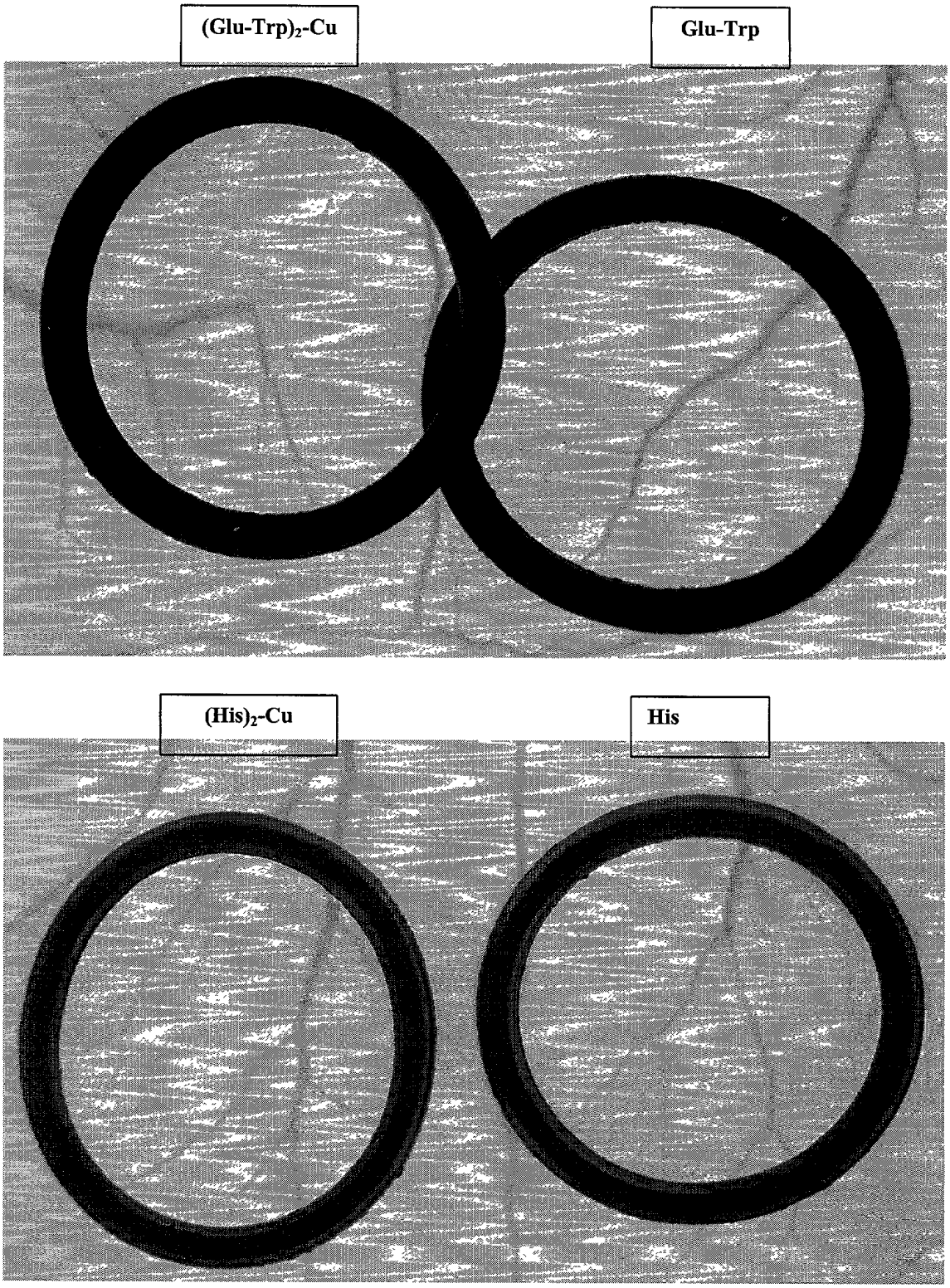
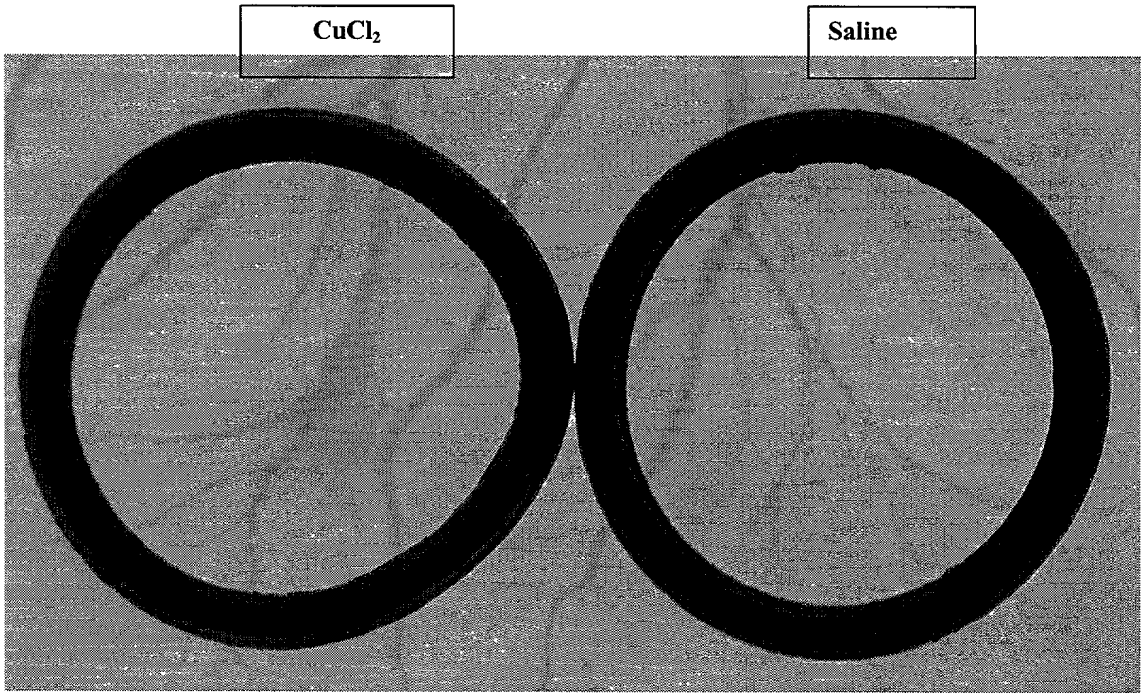


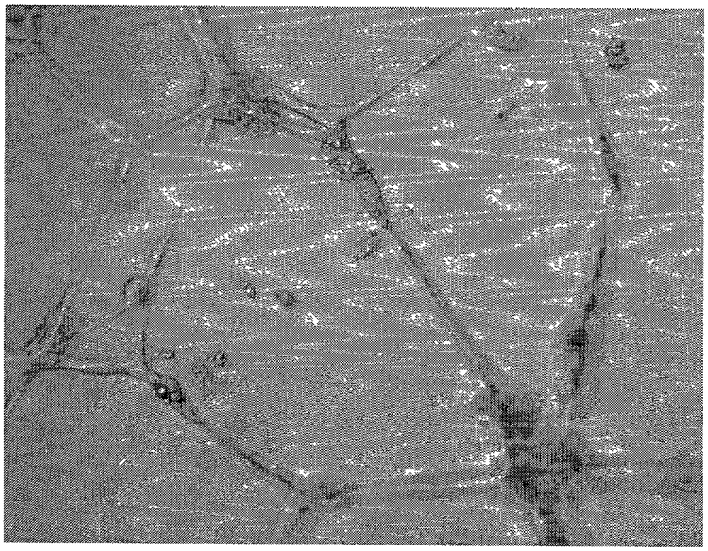
FIG. 6B



(e)

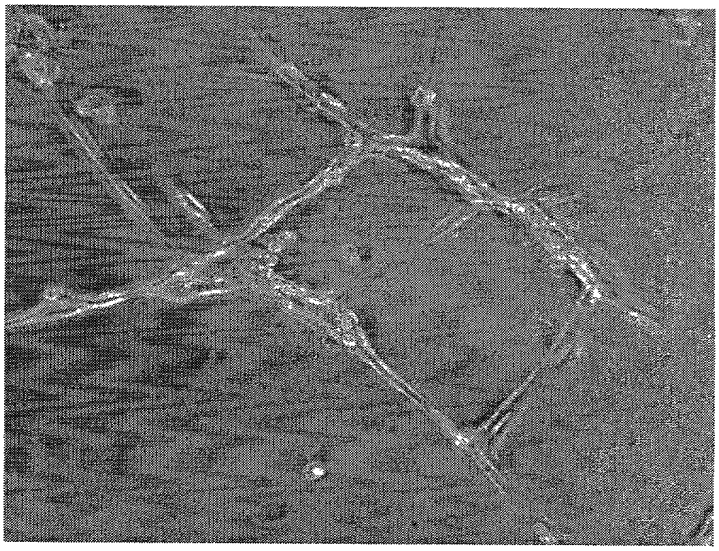
FIG. 6C

Control



(a)

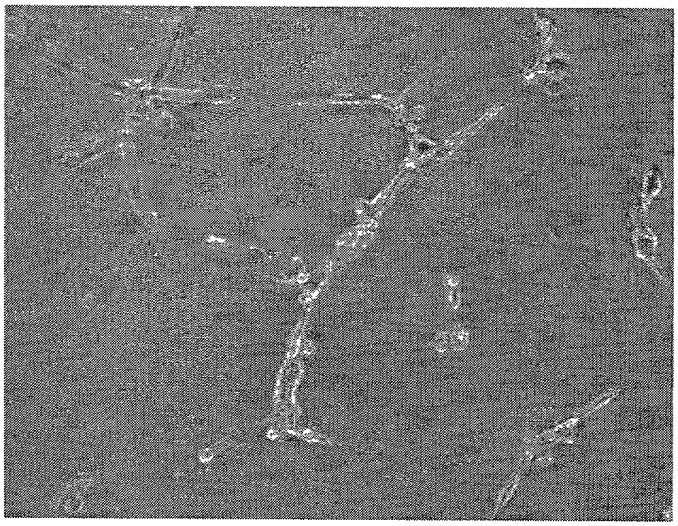
$CuCl_2$



(b)

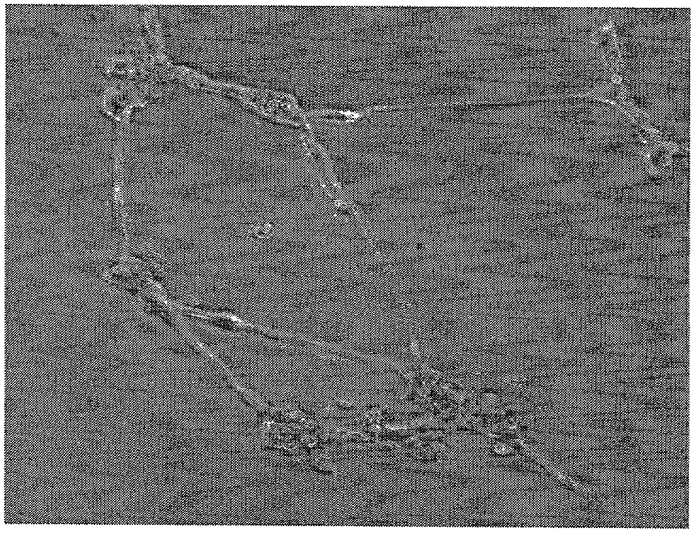
FIG. 7A

Asp-Cu-Asp (Æ-994)



(c)

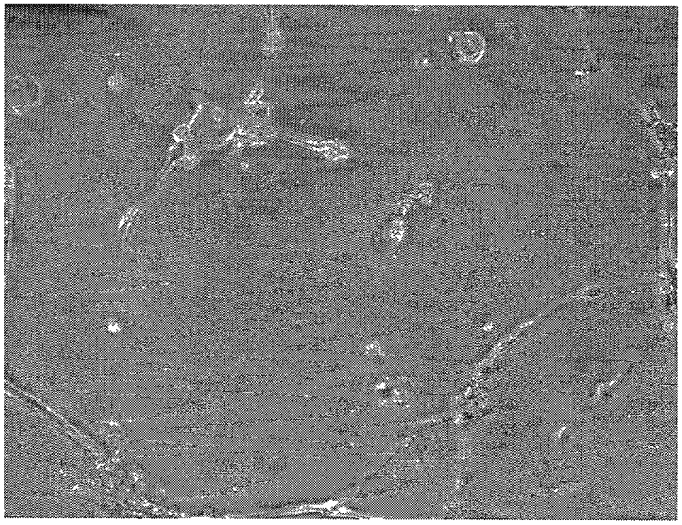
Asp



(d)

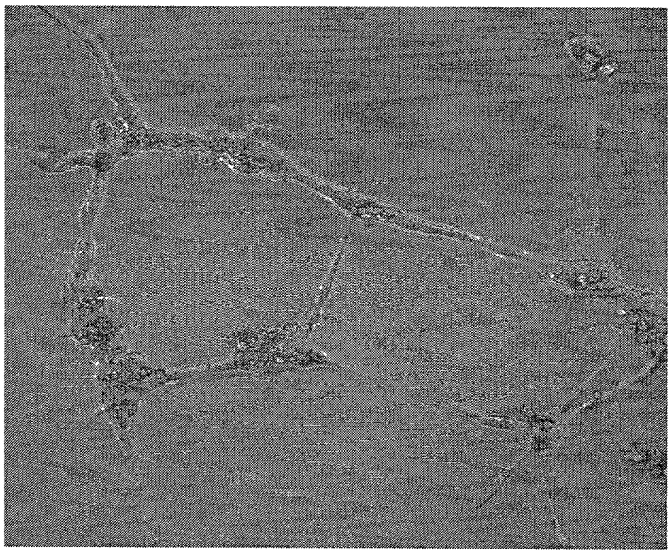
FIG. 7B

Thr-Cu-Thr



(e)

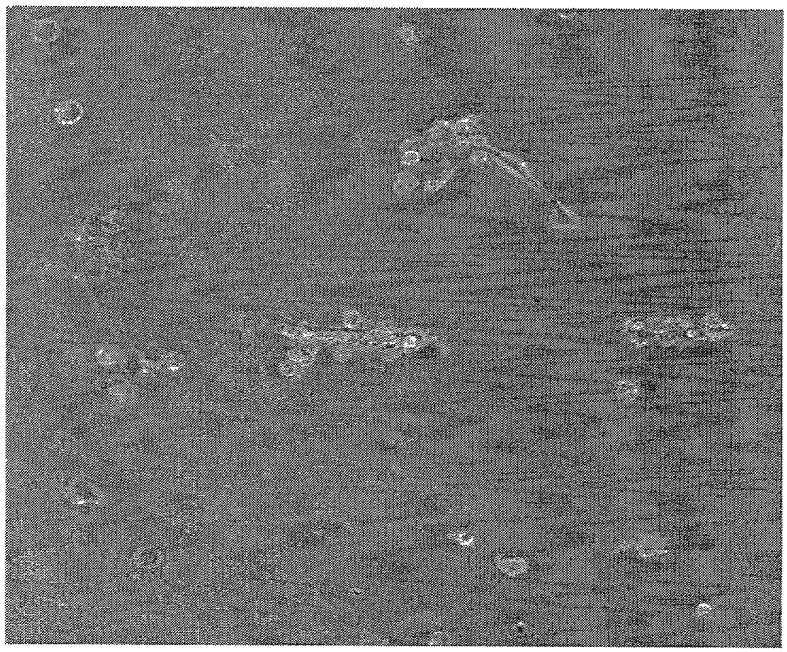
Thr



(f)

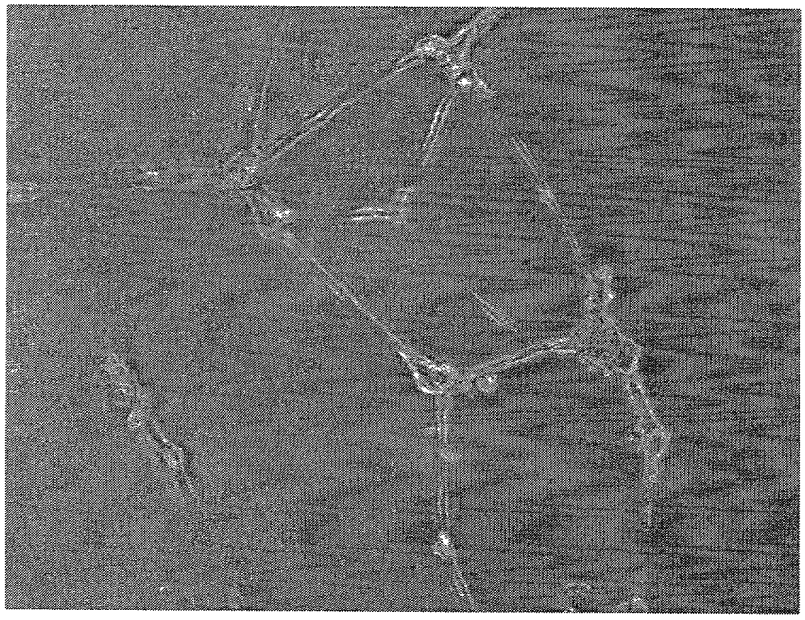
FIG. 7C

$(Glu\text{-}Trp)_2$-Cu



(g)

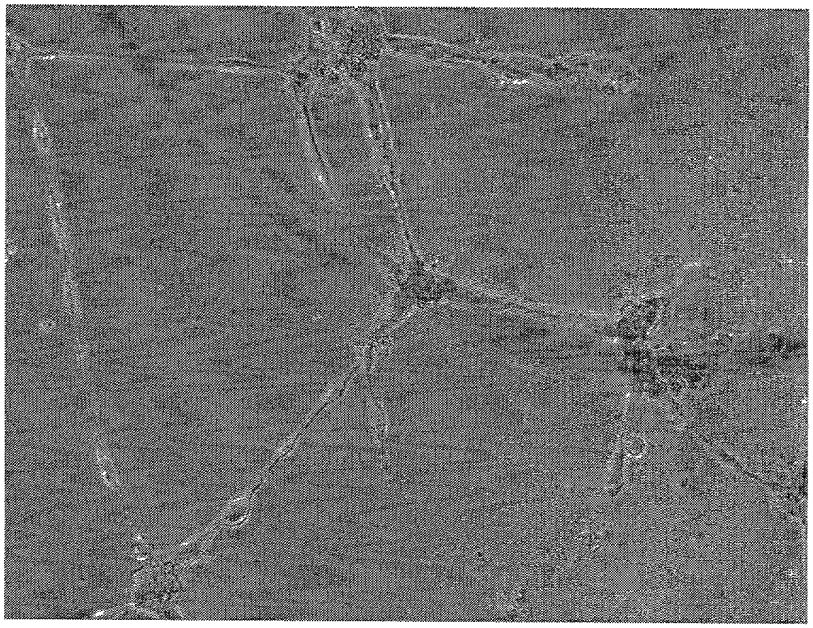
Glu-Trp



(h)

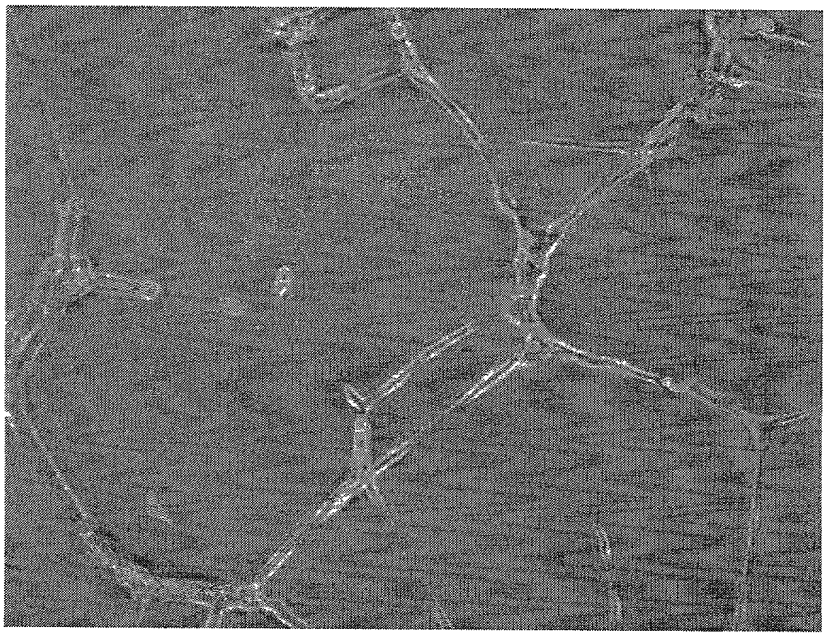
FIG. 7D

His-Cu-His



(i)

His



(j)

FIG. 7E

A: Effect on tumor volume

Saline-treated **Multiple Aa – Cu treated**

B: Effect of vascularization (factor VIII immunochemistry)

Saline-treated **Multiple Amino-acid-Cu- treated**

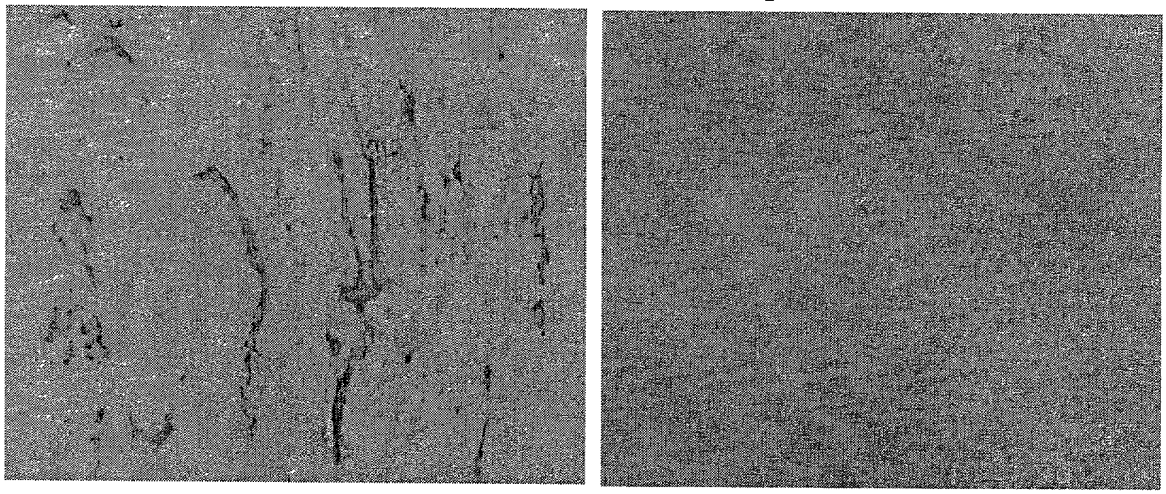


FIG. 8

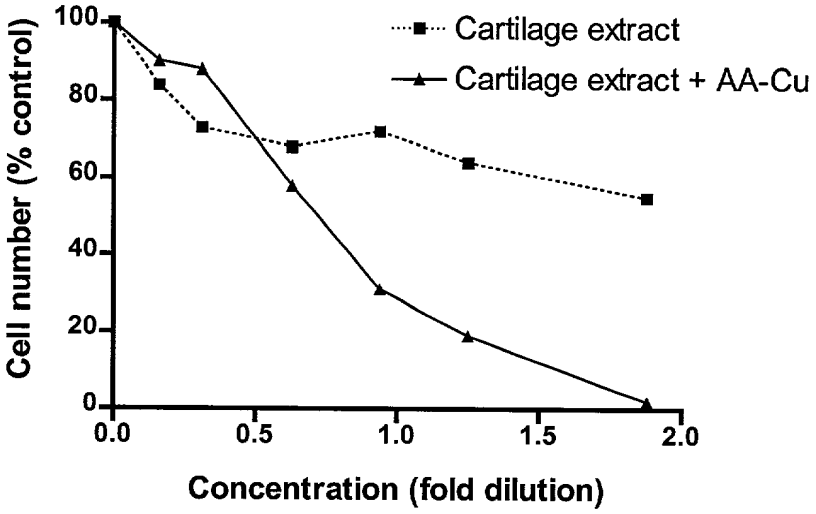

Cartilage extract
Cartilage extract + AA-Cu
Cell number (% control)
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
Concentration (fold dilution)

FIG. 9